8/28/05

In the United States Patent and Trademark Office

Appn. Number: 10/007,489
Appn. Filed: 12/05/2001
Applicant: Elizabeth Gay Frayne
Title: "Microbial Production of Nuclease Resistant DNA, RNA, and Oligo Mixtures"
Examiner: Devesh Khare, PhD, JD
Art Unit:1623

Claims Amendment B

Assistant Commissioner for Patents
Washington, District of Columbia 20231

Sir:

In response to an office action concerning my RCE, I have made changes in the claims. The following Claims Amendment represents a clean amendment. Minor changes have been made to claims 1 and 5 to remove undesired words by the examiner. Claims 2 and 3 were made more lengthy to more completely describe the steps of the invention. Please substitute these recently amended claims for the previous claims of same number. Following is also a marked up version of the claims 1-5 from the prior amendment May, 2005 so the examiner can see the changes. Note only claims 1-5 are pending as claim 6 was withdrawn.

Respectfully submitted,

Elizabeth Frayne

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8/28/05

US Patent and Trademark Office
US Department of Commerce
Appn. Number: 10/007,489
Appn. Filed: 12/05/2001
Applicant: Elizabeth Gay Frayne

Title: "Microbial Production of Phosphorothioate Substituted DNA, RNA, and Oligo Mixtures"

Examiner: Devesh Khare, PhD, JD
Art Unit:1623

RE: Office Action Summary issued Aug. 5th,2005 for Application No. 10/007,489

Dear Sir,

Regards prior art 35 U.S.C. 103(a) rejection concerning citations Sayer et. al. (Directed Mutagen, 46-69,1991) and Nikiforov et al (US Patent 5,518,900). Sayer's work is directed towards a method for site directed mutagenesis using an alpha (S) nucleotide triphosphate to create a phosphorthioate linkage in a newly synthesized DNA strand and Nikiforov's work is directed towards the preparation of single-stranded DNA for use as nucleic acid probes. Both of these methods can be used for the in vitro synthesis of single-stranded DNA. Both methods have the drawback that the template DNA used to direct the synthesis of the single-stranded DNA of interest must be removed. Both methods rely on enzymes in vitro to digest the template DNA. This is not an inexpensive or simple method to generate single stranded DNA except on a limited basis as it requires purified enzymes to both synthesize the DNA and to digest the contaminating template DNA. In addition some sequence knowledge of the DNA of interest is required to design appropriate primers or select restriction enzyme sites to nick the template DNA. To synthesize phosphorothioate DNA by either of these methods would require the use of all four alpha (S) nucleotide triphosphates in purified form. These modified nucleotides are expensive and have only been recently available commercially.

The present invention is very simple and powerful as the only purified component required to synthesize phosphorothiate DNA or RNA is thiophosphate. The bacteria or host cells contain the enzymes necessary to carry out the synthetic reactions for the synthesis of phosphorothioate DNA and RNA. No specialized cell type is required because all cells have the capacity to incorporate thiophosphate into

nucleotide triphosphate pools. Thiophosphate is readily taken up by cells and does not require special permeabilization methods. The compound is also readily incorporated into cells resulting in unprecedented levels of a modified natural substrate. The nucleotide precursor pool can essentially be entirely replaced with the modified phosphate. To produce single stranded phosphorothioate DNA one only needs to grow M13 phage a bacteriophage capable of synthesizing single-stranded DNA in the presence of host cells and culture media containing thiophosphate as a substitute for phosphate. This method allows for the facile and inexpensive production of single-stranded phosphorothioate DNA on a large scale and/or the production of numerous DNA samples on a smaller scale such as from a cloned cDNA library where the sequences are not known. The bacteria naturally performs the final purification step separating single-stranded phosphorothioate DNA from ds DNA by the synthesis of single-stranded phosphorothioate DNA phage that can be easily purified by PEG precipitation.

The present invention is uniquely distinct in how the phosphorothioate DNA is synthesized. The method is very novel in that a micro-organism is used to synthesize the modified DNA by utilizing a modified phosphate in the culture medium.

Please see Claims amendment B for minor objections and amendments for claims 2 and 3. Please under MPEP 707.07(j) the pro se applicant requests that if the Examiner fines patentable subject matter disclosed in this application, but feels that applicant's present claims are not entirely suitable, the Examiner draft one or more allowable claims for the applicant.

Respectfully submitted,

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